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Ny dansk patentansøgning
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Method of improving dough and bread quality
Vor ref: 23720 DK 1

Plougmann, Vingtoft & Partners

A bread improving composition comprising glucose oxidase in combination with oxidases or hydrolases such as for example lipase is disclosed in EP 468 731 A1. There is obtained bread of a sufficient volume. However, according to this prior art document the use of lipase alone did not result in bread of a satisfactory quality.

WO-94/04035 discloses a method of improving the properties of a dough (with and without added fat) and/or a baked product made from the dough by adding a lipase (EC 3.1.1.3) of microbial origin to the dough. The use of the microbial lipase resulted in an increased volume and improved softness of the baked product. Furthermore, an anti-staling effect was found.

EP 585 988 A1 discloses a bread improver composition comprising at least one lipase, at least one hemicellulase and at least one amylase. Baking experiments showed that the use of lipase alone in a dough without added fat resulted in a reduced volume of the baked product whereas no volume effect was observed when lipase was used in a dough containing added fat.

WO98/45453 discloses the use of a lipase form *Aspergillus niger* for improvement of bread crumb structure. However, this enzyme did not have significant improving effects on bread volume and softness.

From the prior art it can thus be derived that the effects of lipases when used as dough additives are highly variable in respect of antistaling or crumb firmness retardation and improvement of bread volume.

A significant effect of the lipase disclosed in WO98/45453 is that it, in addition to its triglyceride hydrolysing effect, is capable of hydrolysing polar glycolipids present in flour, such as e.g. digalatosyldiglyceride (DGDG). It was hypothesised that the bread crumb structure improving effect might be associated with this latter effect. It was also shown that the *Aspergillus niger* lipase did not have a phospholipid hydrolysing effect.

It is known to use phospholipases for improvement of bread quality. Thus, JP-82-66213 discloses the use of phospholipase C and a lysophospholipase for improvement of frozen doughs, EP 575 133 A discloses the use of phospholipase A1 to improve handling properties of doughs. JP-60-078529 describes the use of a phospholipase A to improve

the mechanical properties of wheat flour doughs and noodles and EP 109 244 A discloses that phospholipase A can be used to improve the properties of doughs.

Whereas the addition of lipases or phospholipases to flour doughs to improve the mechanical properties of the doughs and/or the quality of the finished baked products is known in the art, it is a significant problem that the amount of lipid substrates for the respective enzymes present in flour is limited. The substrate for lipases in a wheat flour dough is the endogenous lipids of which about 50% are nonpolar lipids and 50% are polar glycolipids and phospholipids.

Several of the lipases presently used in the baking industry including those disclosed in EP 585 998 and WO 94/04035 are only active against the nonpolar lipid fraction resulting in the formation of free fatty acids and glycerol and to a less extent, mono- or diglycerides. The beneficial effects on dough or bread quality is, however, rather limited as free fatty acids may have an adverse effect on bread quality. The fungal lipase disclosed in WO98/45453 has, in addition to its effect on acylglycerols, a certain hydrolysing effect on the polar glycolipids.

Additionally, as it is mentioned above, the use of phospholipases A and C has been suggested as dough and/or bread improving additives. The enzymatic effect of such phospholipases is that the phospholipids present in the dough is converted into the corresponding lysophospholipids which are known to be effective emulsifying agents. However, the amount of endogeneous phospholipids in bread doughs is relatively small and therefore the dough and bread improving effect of adding enzymes that are selectively active against phospholipids will be limited.

There is therefore a need for a dough and/or bread improving enzyme that is capable of hydrolysing substantially all of the lipid types present in flour doughs, i.e. the nonpolar acylglycerols and the polar phospholipids and glycolipids. The present invention is based on the discovery of lipolytically active enzymes that are capable of utilising all of these lipids simultaneously as substrates and it has been found that the addition of such an enzyme to a dough results in significant improvements of dough stability and strength and the handling properties of doughs and in improved quality of the baked bread products in terms of a significant enhancement of bread volume, crumb structure, crumb appearance, and colour and of the softness of the bread. A particularly interesting and important

aspect of these novel enzymes is that they may have preference for polar lipids implying that the adverse effects that have been observed for acylglycerol hydrolysing lipases can be controlled by selecting enzymes preferentially hydrolysing polar lipids.

It has also been found that the dough and bread improving effects of the enzymes according to the invention can be further enhanced by adding glycolipids and/or phospholipids to the dough, e.g. in the form of cereal lipids including oat oil.

SUMMARY OF THE INVENTION

Accordingly, the invention pertains in a first aspect to a method of preparing a flour dough, said method comprising adding to the dough components an enzyme that, under dough conditions, is capable of hydrolysing a nonpolar lipid, a glycolipid and a phospholipid, or a composition containing said enzyme, and mixing the dough components to obtain the dough. Any of the lipid substrates for the enzyme can be lipids naturally present in the flour or they may added to the dough.

In a further aspect, there is provided a dough improving composition comprising an enzyme that, under dough conditions, is capable of hydrolysing a nonpolar lipid, a glycolipid and a phospholipid, and optionally at least one further dough component. The further dough component may e.g. be any other enzymes that has an improving effect on the dough properties and/or the quality of a baked product made from the dough.

In still further aspects the invention relates to a dough that is obtained by the method of the invention and baked products that is obtained by baking such a dough, and noodle and pasta products prepared in accordance with the invention.

DETAILED DISCLOSURE OF INVENTION

A primary objective of the present invention is to provide a method to improve the properties of flour based doughs and products made from such doughs. This is, in respect of baked products, achieved by providing a method for preparing baked products which have highly desirable characteristics with respect to bread volume, crumb structure and ap-

pearance and which additionally have an extended shelf life as reflected in an enhanced softness, i.e. the staling of the baked products is retarded relative to a baked product made without use of the enzyme of the invention. Although it is presently preferred to use the method for the manufacturing of yeast leavened bread products such as bread loaves, rolls or toast bread, the use of the method for any other types of doughs and dough based products such as noodle and pasta products and cakes, the quality of which can be improved by the addition of the enzymes of the invention, is also contemplated.

The present method comprises as an essential step that an effective amount of an enzyme that, under dough conditions, is capable of hydrolysing a nonpolar lipid, a glycolipid and a phospholipid, or a composition containing said enzyme is added to the dough either directly to an already mixed dough or as a component of one or more dough components.

In the present context, the expression "an effective amount" is used generally to describe an amount of the enzyme which is sufficient to effect, under dough conditions, detectable hydrolysis of triglycerides, phospolipids and glycolipids present in the dough. Examples of analytical methods permitting detection of these hydrolytic activities are given in the below examples. More specifically, the expression may relate to an amount which does not only result in detectable hydrolysis of the above lipid substrates, but which in addition results in the formation of enzymatic end products at a level which results in improved properties of the dough such as a significantly improved stickiness score and/or extensibility score which can be ascribed to the addition of the enzyme, or, if the dough is baked, in an improved quality of the baked product such as enhanced bread volume, enhanced softness or improved crumb structure.

The enzyme of the invention can be described as a multifunctional enzyme that is capable of simultaneously hydrolysing acylglycerols (glycerides) (i.e. it has an esterase activity generally associated with the class of enzymes referred to as lipases (EC 3.1.1.3)), phospholipids and glycolipids such as galactolipids. Accordingly, the enzyme has i.a. a hydrolysing activity that is associated with a variety of enzymes generally referred to as phospholipases. Phospholipids are cleaved in two different ways by two groups of enzymes, one of which is included in the group of lipases and which include phospholipase A_1 and phospholipase A_2, and phosphodiesterases (phospholipases C and D). The enzyme of the invention may have any of these phopholipase activities.

It has been found that enzymes having the hydrolytic characteristics of the enzyme according to the invention may have different affinities for fatty acid moieties in the lipid substrate implying that the enzyme of the invention may preferentially hydrolyse lipids containing short chain fatty acids such as C_4 to C_{10} fatty acids or it may preferentially hydrolyse lipids having long chain fatty groups such as C_{12} to C_{20} fatty acids. Enzymes having preference for long chain fatty acid groups may e.g. be particularly useful in doughs where butter fat or other lipids containing butyric acid groups, as it is a known problem that free butyric acid may give rise to undesirable taste and flavour.

One implication of this selective substrate profile is that an enzyme can be selected which are particularly active in a given dough depending on the recipe and the lipid content hereof.

The enzyme having the properties as defined herein may be derived from a variety of sources including plants, animals and microorganisms such as bacterial and fungal species including yeast species. The enzyme of the invention may be derived from an organism that naturally produces the enzyme or it may be produced recombinantly by transforming an appropriate host cell with a gene coding for the enzyme. The enzyme can be an enzyme that comprises in itself active sites for all of its enzyme activities, but it is also possible to construct hybrid enzymes having the enzyme activities as defined herein by synthesis or by using recombinant DNA technology.

Presently preferred enzymes for use in the invention are Lipase SP 972 and Lipase SP 979, the effect of which are described in details in the below examples.

Most cereal flours contain nonpolar lipids including triglycerides and polar lipids including phospholipids and glycolipids which can serve as substrates for the enzyme of the invention. Accordingly, in one embodiment of the method, at least one of the nonpolar lipid, the glycolipid such as a galactolipid including digalactosyldiglyceride (DGDG), and the phospholipid such as phosphatidylcholine (PC) is a naturally occurring (or endogenous) lipid component occurring in the flour used for the dough.

However, a flour dough may not contain sufficient amounts of all of the lipid substrates for the enzyme of the invention. It is therefore within the scope of the invention to supplement the dough with at least one of a nonpolar lipid, a glycolipid and a phospholipid to provide

sufficient substrates for the enzyme. It will be appreciated that the expression "sufficient substrate" implies that none of the three main types of lipid substrates is limiting for obtaining a dough improving or baked product improving effect as described above.

The supplementary lipid substrate for the enzyme of the invention may be a nonpolar lipid such as an acylglycerol. In accordance with the invention a variety of such lipids can be used such as e.g. vegetable oils, vegetable fats, animal oils, animal fats such as e.g. butterfat, and shortening. In this connection, a particularly useful lipid is an oil or a fat derived from cereals such as oat oil. Oat oil typically contains, in addition to triglycerides, 5-25% phospholipids and 5-12% glycolipids. Oat oil can be fractioned to yield fractions having a high content of polar lipids.

It is thus one aspect of the method of the invention that one or more phospholipids can be added to the dough. In this connection, useful phospholipids include phosphatidylinositol (PI), phosphatidylglycerol (PG), phosphatidylcholine (PC), lecithin and phosphatidylethanolamine (PE).

In accordance with the invention, the enzyme is added in an amount which is in the range of 10 to 100,000 LUT/kg flour or in the range of 10 to 100,000 PLU/kg flour, the unit designations are those defined in the below examples, such as a range of 50 to 50,000 LUT or PLU/kg flour including a range of 100 to 10,000 LUT/kg flour or 100 to 10,000 PLU/kg flour.

Another objective of the invention is to provide a method for obtaining a baked product having improved quality characteristics as defined above. Accordingly, in one embodiment, the dough being prepared by the method of the invention is a bread dough and the method comprises as a further step that the dough is baked to obtain a baked product. One particularly desired property of baked bread products is a high specific volume as defined in the examples. Accordingly, the addition of the enzyme of the invention preferably results in an increase of the specific volume of the baked product that is at least 10%, relative to a baked product made under identical conditions except that the enzyme is not added. More preferably, the increase of the specific volume is at least 20% such as at least 30%, e.g. at least 40%.

It is another objective of the invention to provide pasta doughs, noodle doughs and cake doughs or batters and finished products made from such doughs or batters.

It is known in the art that enzymes other than lipases may contribute to improved dough properties and quality of baked produts. It is within the scope of the invention that, in addition to the enzyme of the invention, at least one further enzyme is added to the dough. Such further enzymes include starch degrading enzymes such as endo- or exoamylases, pullulanases, debranching enzymes, hemicellulases including xylanases, cellulases and oxidoreductases, e.g. glucose oxidase, lipases, phospholipases and hexose oxidase.

It has been found that the enzyme of the invention may be particularly active against certain glycolipids such as e.g. galactolipids including digalactodiglyceride (DGDG) which is converted into digalactomonoglyceride (DGMG) that is an effective surfactant. In useful embodiments, the enzyme of the invention is therefore an enzyme that is capable of hydrolysing at least 25% of DGDG initially present in the dough and preferably at least 50% of the DGDG is hydrolysed such as at least 60% or at least 75% hereof.

Another useful lipid substrate for the present enzyme is the phosholipid, phosphatidyl choline (PC). Thus, in useful embodiments the enzyme is capable of degrading at least 25%, preferably at least 50% including at least 60% such as at least 75% of the PC initially present in the dough.

It is one advantageous aspect of the present enzymes that they may be more hydrolytically active against certain of the lipid substrate types as defined above than they are against other types. The enzymes may thus be relatively more active against polar lipids than they are against nonpolar triglycerides. This can be illustrated by analysing the amount of any of the lipid substrate types being hydrolysed and then construct a curve describing the relationship between the hydrolysis of any pairs of lipid substrates, e.g. triglycerides vs. glycolipid or triglycerides vs. phospholipids.

In specific embodiments, the enzyme of the invention is characterised in that the relationship between the ability of the enzyme to hydrolyse triglycerides and the ability to hydrolyse glycolipids can be described as a curve having a slope which is at least 1.0, such at least1.5 or at least 2.0 or in that the relationship between the ability of the enzyme to hydrolyse triglycerides and the ability to hydrolyse phospholipids can be described as a

curve having a slope which is at least 0.1, such as e.g. at least 0.2, including at least 0.5 or at least 1.0.

In a further aspect, the invention provides a dough improving composition comprising an enzyme that, under dough conditions, is capable of hydrolysing a nonpolar lipid, a glycolipid and a phospholipid, and optionally at least one further dough component. Such a further dough component can e.g. be a further enzyme as defined above, including lipases or phospholipases not having the substrate profile of the present enzyme. Other suitable dough components which can be incorporated in the composition include cereal flours such as wheat flour, rice flour and corn flour, yeast, chemical leavening agents, dough strengthening agents such as oxidoreductases and ascorbates, emulsifiers, sugars, acylglycerols of the types mentioned above, phospholipids such as soy lecithin and egg lecithin, glycolipids and salts.

In further aspects of the invention there is provided a method of preparing a dough as defined above wherein the enzyme is added in a composition as described above and a dough that is obtained in accordance with the methods of the invention. Such a dough can be a fresh dough, optionally packaged in a controlled atmosphere to keep it fresh or it may be a frozen dough.

The invention is further illustrated in the following non-limiting examples and the drawings where:

Fig. 1 illustrates the effect on bread crumb structure of a commercial lipase, GRINDAMYL™ EXEL 16 (200 ppm) (bread rolls to the left), Lipase SP 972 (1000, 2500 and 5000 LUT/kg flour, respectively) (rolls 2-4) as compared to a control without addition of lipase (rolls to the right);

Fig. 2 shows the effect on bread volume and crumb structure of addition of oat lipid alone (bread to the left), oat lipid + 1010 ppm SP 979 (bread in the middle) and oat lipid + 200 ppm GRINDAMYL™ EXEL 16 (bread to the right),

Fig. 3 illustrates the relationship between the ability of Lipase SP 972 and Lipase SP 979 to hydrolyse triglycerides and their ability to hydrolyse glycolipids, and

Fig. 4 illustrates the relationship between the ability of Lipase SP 972 and Lipase SP 979 to hydrolyse triglycerides and the ability to hydrolyse phospholipids.

EXAMPLES

Materials and methods

1. Enzymes used

Enzyme preparations designated SP 972 and SP 979, respectively, were obtained from Novo Nordisk, Bagsvaerd, Denmark. The enzyme designations are those used by Novo Nordisk.

The following lipases were used as references: GRINDAMYL™ EXEL16 (Danisco Ingredients, Brabrand, Denmark) and Stalingase™ (Gist-brocades, Delfts, the Netherlands).

2. Lipase activity using tributyrin as substrate (LUT and LIPU)

Lipase activity based on the use of tributyrin as substrate was measured as described in Food Chemical Codex, 4th edition, National Academy Press, 1996, p. 803 with the modification that samples were dissolved in deionised water instead of glycine buffer and that the pH-stat set point was 5.5 instead of 7.

1 LUT is defined as the amount of enzyme which can liberate 2 µmol butyric acid per min. under the assay conditions. 1 LIPU is defined as the amount of enzyme that can liberate 1 µmol butyric acid per min. under the assay conditions.

3. Lipase assay based on the use of sunflower oil as substrate (LUSol, pH 6.5)

Reagents: 8.4 g gum arabic is dissolved in 100 ml demineralised water and 100 ml 30 mM $CaCl_2$ is added. 36 ml sunflower oil is added slowly under mixing using an Ultra Turrax™ mixer at 20,000 rpm to obtain an emulsion.

Assay: 20 ml of sunflower oil emulsion in a beaker is equilibrated at 30°C for 5 min. and pH adjusted to 6.3-6.5 using a pH-stat. 2 ml of enzyme preparation is added and 0.05 N NaOH is added continuously while keeping the pH at 6.5 for 10 min. The slope of the curve for addition of 0.05 N NaOH as a function of time is calculated.

1 LUSol is defined as the quantity of enzyme that can liberate 1 µmol fatty acid per min. under assay conditions.

3. Phospolipase assay (PLU, pH 8.0)

Substrate: 8 g of lecithin powder (Metarin P,074783) is dissolved in 150 ml water under heating to 40-50°C. 40 ml of 50 mM $CaCl_2$ is added and water is added to 200 ml. The substrate mixture is homogenised for 1 min. using an Ultra Turrax™ mixer at 20,000 rpm.

Assay: 20.0 ml of substrate is transferred to a beaker and equilibrated at 30°C for 5 min. and pH adjusted to 8.0 and 2 ml of enzyme preparation is added followed by continuously adding 0.05 N NaOH for 10 minutes while keeping the pH at 8.0.

The slope of the curve for the addition of 0.05 N NaOH as a function of time is calculated. 1 PLU is defined as the amount of enzyme which can liberate 1 µmol of fatty acid per min. under assay conditions.

4. Baking test (Toast bread)

2000 g of Danish reform flour, 30 g of dry yeast, 30 g of sugar, 30 g of salt and water at 400 Brabender units (BU) + 3% was kneaded using a Hobart™ Mixer with hook for 2 min. at low speed and 10 min. at high speed. Dough temperature after mixing was 25°C. Resting time was 10 min. at 30°C. The dough was scaled at 750 g per dough and rested again for 5 min. at 33°C and 85% RH. Moulding was carried out using a Glimik™ moulder. The doughs were proofed in tins for 50 min. at 33°C and baked in Wachtel™ oven for 40 min. at 220°C and steam injection for 16 sec. After cooling, the bread was scaled and the volume of the bread measured using the rape seed displacement method.

The crumb was also evaluated subjectively on a scale 1 to 10, where 1 = coarse structure and 10 = homogeneous structure.

Three loaves baked in tins provided with lids were stored at 20°C and used for firmness measurements.

5. Firmness measurement

Firmness of bread was measured using an Instron™ UTM model 4301 connected to a computer. Conditions for measurements were as follows:

Load cell	Max 100 N
Piston diameter	50 mm
Cross Head Speed	200 mm/min.
Compression	25%
Bread slice thickness	11 mm

The force is converted to N/dm^2. The results were calculated as an average of 10 bread slices per loaf.

6. Baking test (Hard crust rolls)

1500 g of Danish reform flour, 90 g of compressed yeast, 24 g sugar, 24 g salt and water at 400 Brabrender units + 2% were kneaded using a Hobart™ mixer with hook for 2 min. at low speed and 6 min. at high speed. The dough temperature was 26°C. The dough was scaled at 1350 g and was rested for 10 min. at 30°C and moulded using a Fortuna™ moulder. The moulded dough was proofed for 45 min. at 34°C and baked in a Bago™ oven for 18 min. at 220°C and with steam for 12 sec. After cooling, the rolls were scaled and the volume of the rolls was measured using the rape seed displacement method.

The specific volume of the rolls was calculated as follows:

$$\text{Specific volume} = \frac{\text{Volume of the bread, ml}}{\text{weight of bread, g}}$$

7. Mini baking test

50 g of Danish reform flour, 10 g dry yeast, 0.8 g sugar, 0.8 g salt, 70 ppm ascorbic acid and water at 400 Brabender units was kneaded using a 50 g Brabrender™ mixing bowl for 5 min. at 30°C. Resting time was 10 min. at 34°C. The dough was scaled at 15 g per dough and moulded using a device where the dough is rolled between a wooden plate and a plexiglass frame. The doughs were proofed in tins for 45 min. at 34°C and baked in a Voss™ household oven for 8 min. at 225°C. Following baking, the miniloaves were cooled to ambient temperature and after 20 min. the loaves were scaled and the volume determined by the rape seed displacement method. The loaves were cut and the crumb and crust evaluated.

8. Lipid extraction and fatty acid analysis

20 g of fully proofed dough was frozen immediately and freeze-dried. The freeze-dried dough was milled using a coffee bean mill and passed through a 800 µm screen. 2 g of freeze-dried dough was scaled using a 15 ml centrifuge tube with screw cap. 10 ml of water-saturated butanol (WSB) was added. The centrifuge tube was placed in a boiling water-bath for 10 min. The tubes were placed in a Rotamix™ apparatus and turned at 45 rpm for 20 min. at ambient temperature and subsequently placed in a boiling water-bath for 10 min and turned on the Rotamix™ apparatus for 30 min. at ambient temperature. The tubes were centrifuged at 3,500 g for 5 min. 5 ml of supernatant was transferred into a vial. WSB was evaporated to dryness under a stream of nitrogen.

The free fatty acids in the extract were analysed as Cu salts in isooctane measured at 715 nm and quantified according to a calibration curve based on oleic acid (Kwon, D.Y. and J.S. Rhee (1986). A Simple and Rapid Colourimetric Method for Determination of Free Fatty Acids for Lipase Assay, JAOCS 63:89).

9. HPLC analysis

Column: LiChrospher™ 100 DIOL 5 µm (Merck art. 16152) 250 x 4.0 id with water jacket, 50°C.

Mobile phase:

A: heptane/isopropanol/butanol/tetrahydrofuran/isooctane/H_2O^*, 64.5/17.5/7/5/5/1

B: isopropanol/butanol/tetrahydrofuran/isooctane/H_2O^*, 730/7/5/5/10

* 1 mmol trifluroacetic acid/l of mobile phase
(pH = 6.6 adjusted with NH_3)

Pump: Waters™ 510 + Gradient controller

Gradient:

Flow: ml/min.	Time: min.	% A	% B
1.0	0	100	0
1.0	25	0	100
1.0	30	0	100
1.0	35	100	0
1.0	40	100	0

Detector: CUNOW™ DDL21 (evaporative light scattering) (temp: 100°C, voltage: 600, air flow: 6.0 l/min.)

Injector: Hewlett Packard™ 1050, injection volume 50 µl

Sample preparation: The wheat lipid was dissolved in 5 ml $CHCl_3/CH_3OH$ (75:25), sonicated for 10 min. and filtered through a filter having a pore size of 0.45 µm.

Calculation: Calibration curve for PC (lecithin standard from International Lecithin and Phospholipid Society)

Reference: Arnoldsson, K.C. and P. Kaufmann (1996), Chromatographia 38:317

10. Gas chromatography

Perkin Elmer™ 8420 Capillary Gas Chromatograph equipped with a WCOT fused silica column, 12.5 x 0.25 mm ID and 0.1 µm 5% phenylmethyl silicone (CP Sil 8 CB from Chrompack) was used with helium as carrier under the following conditions:

Detector: FID, 385°C

Oven programme:

Programme No.	1	2	3	4
Oven temperature, °C	80	200	240	360
Isothermal time, min.	2	0	0	10
Temperature rate, °C/min.	20	10	12	

Sample preparation: 50 mg wheat lipid was dissolved in 12 ml heptane:pyridine, 2:1 containing 2 mg/ml heptadecane as an internal standard. 500 µl was transferred to a crimp vial and 100 µl MSTFA (N-Methyl-N-trimethylsilyl-trifluoroacetamide) was added and reacted for 15 min. at 90°C).

Calculation: Response factors for mono-, di- and triglycerides and free fatty acids were determined from reference mixtures of these components. Based on these response factors the content of mono-, di- and triglycerides and free fatty acids in wheat lipids were calculated.

11. Enzymatic activities of enzymes used in the following examples:

Enzyme	LIPU/g	LUSol/g	PLU/g
Lipase SP 979	0	65	1984
Lipase SP 972	280	40	2450
GRINDAMYL EXEL 16	3000	450	0
Stalingase, # 1867, purified	42.5	24.7	5
Lipase 3 (2524-120)	4600	2910	24

EXAMPLE 1

The effect of SP 972 and GRINDAMYL™ EXEL 16 in hard crust rolls

The rolls were baked and tested according to the procedures described above. The results are summarised in the below Table 1.1:

Table 1.1. Specific volume and crumb score (1-10)

Enzyme	Specific volume, ml/g	Crumb score
Control w/o enzyme	5.33	4
200 ppm EXEL 16	5.65	7
1000 LUT/kg SP972	5.70	6
2500 LUT/kg SP972	5.99	8
5000 LUT/kg SP972	5.46	8

Fully proofed doughs from this baking test were extracted and the content of free fatty acids was measured. The results are summarised in Table 1.2:

Table 1.2. Content of free fatty acid in proofed doughs

Enzyme	% free fatty acids
Control w/o enzyme	0.229
200 ppm EXEL 16	0.303
1000 LUT/kg SP972	0.349
2500 LUT/kg SP972	0.324
5000 LUT/kg SP972	0.364

These results indicate a clear effect of Lipase SP 972 on the fatty acid formation in doughs.

Additionally, the bread volume is significantly improved by the addition of Lipase 972 and this enzyme confers a more white and homogeneous crumb structure as it is illustrated in Fig. 1.

EXAMPLE 2

The content of fatty acids and polar lipids in proofed doughs supplemented with lipase

Five test doughs (1-5) were mixed using a 50 g Brabender™ Farinograph at 30°C for 5 min. and proofed at 34°C for 60 min. followed immediately by freezing and freeze-drying.

The compositions of the test doughs are summarised in Table 2.1:

Table 2.1. Composition of test doughs

Component	Test dough 1 (control)	Test dough 2	Test dough 3	Test dough 4	Test dough 5
Reform flour, g	50	50	50	50	50
Dry yeast, g	0.38	0.38	0.38	0.38	0.38
Salt, g	0.75	0.75	0.75	0.75	0.75
Water at 500 BU (54%), ml	27	26.19	24.95	22.94	18.87
SP972 (580 LUT/ml), ml	0	0.813	2.05	4.06	8.13
LUT/kg flour	0	894	2236	4472	8943

The content of fatty acids were analysed by GC. The results of these analyses are summarised in Table 2.2:

Table 2.2. Content of fatty acids in test doughs 1-5

Test dough	% fatty acids
1	0.180
2	0.394
3	0.410
4	0.469
5	0.522

Additionally, the content of polar lipids in the control dough (test dough 1) and test dough 5 were analysed by HPLC as described above. The results are summarised in Table 2.3:

Table 2.3. Content of polar lipids in test doughs containing 0 and 8943 LUT lipase, respectively

Polar lipid	% polar lipid in control dough	% polar lipid in test dough 5
Acylated phosphatidylethanoleamine (APE)	0.044	0.056
Digalacosyldiglyceride (DGDG)	0.209	0.022
Phosphatidylcholine (PC)	0.051	0.003
Digalactosylmonoglyceride (DGMG)	0.015	0.103
Lysophosphatidylcholine (LPC)	0.268	0.301

The above results indicate a clear effect of Lipase SP 972 on the formation of fatty acids. Even at the lowest dosage of 894 LUT/kg flour, a significant increase in fatty acid content in the test dough was observed. The HPLC analyses indicate a remarkable effect in respect of hydrolysis of phospholipids and glycolipids.

Accordingly, these experiments demonstrates that the SP 972 Lipase is capable of utilising glycerides, phospolipids and glycolipids as substrate.

EXAMPLE 3

The effect of Lipase SP 972 alone and in combination with soy lecithin on the content of fatty acids and polar lipids in hard crust roll dough and the quality of the baked rolls

Lipase SP 972 was tested for its activity alone and in combination with soy lecithin on the quality of hard crust rolls. A commercial lipase product, GRINDAMYL™ EXEL 16 (EXEL 16) and a commercial DATEM emulsifier, Panodan™ A 2020 were tested for comparison.

The used amounts of enzyme and emulsifier additives and the quality characteristics of the respective baked rolls are summarised in Table 3.1:

Table 3.1. Enzyme and emulsifier additions and bread quality

	Specific volume, ml/g	Crumb score (1-10)	Crust score (1-10)
Control	5.95	3	3
0.2% Panodan A 2020	6.14	N.D[1]	N.D[1]
750 LUT/kg SP 972	6.77	8	7
1500 LUT/kg SP 972	6.65	7	6
750 LUT/kg SP 972 + 0.5% lecithin	6.94	5	8
1500 LUT/kg SP 972 + 0.5% lecithin	7.2	5	7
900 LUT/kg EXEL 16	6.38	7	5
1800 LUT/kg EXEL 16	6.2	6	6
900 LUT/kg EXEL 16 + 0.5% lecithin	6.3	5	7
1800 LUT/kg EXEL 16 + 0.5% lecithin	6.35	7	7

[1]Not determined

Baking experiments using Lipase SP 972 as compared to GRINDAMYL™ EXEL 16 or Panodan A 2020 emulsifier showed excellent baking performance of SP 972 both alone and in combination with soy lecithin. This effect is significantly better than that of the commercial lipase, GRINDAMYL™ EXEL 16.

Lipids extracted from the dough were analysed for free fatty acids and polar lipids as described above. The results of these analyses are summarised in Tables 3.2 and 3.3:

Table 3.2. Content of free fatty acids in doughs containing an emulsifier, Lipase SP 972 alone or in combination with lecithin or GRINDAMYL™ EXEL 26 alone or in combination with lecithin

	Free fatty acids, wt%
0.2% Panodan A 2020	0.129
750 LUT/kg SP 972	0.148
1500 LUT/kg SP 972	0.235
750 LUT/kg SP 972 + 0.5% lecithin	0.259
1500 LUT/kg SP 972 + 0.5% lecithin	0.280
900 LUT/kg EXEL 16	0.263
1800 LUT/kg EXEL 16	0.330
900 LUT/kg EXEL 16 + 0.5% lecithin	0.337
1800 LUT/kg EXEL 16 + 0.5% lecithin	0.346

Table 3.3. The content of polar lipids (wt%) in doughs supplemented with Lipase 972 alone or in combination with lecithin or GRINDAMYL™ EXEL 16 alone or in combination with lecithin

	DGDG	DGMG	PC	LPC
750 LUT/kg SP 972	0.089	0.083	0.023	0.303
750 LUT/kg SP 972 + 0.5% lecithin	0.116	0.076	0.031	0.326
900 LUT/kg EXEL 16	0.233	0.018	0.067	0.285
900 LUT/kg EXEL 16 + 0.5% lecithin	0.240	0.015	0.015	0.290

The above results indicate that GRINDAMYL™ EXEL 16 has no effect on hydrolysis of DGDG and PC. It can therefore be concluded that modification of both phospholipids and glycolipids in the dough effected by Lipase SP 972 is of importance for the bread improving effect of Lipase 972 as compared to the commercial lipase, GRINDAMYL™ EXEL 16.

EXAMPLE 4

The enzymatic activity of Lipase SP 972 in a dough supplemented with oat lipid

Lipase SP 972 was tested in a model dough supplemented with an oat lipid fraction, 2133-18-1. The recipe of the dough was as follows:

Table 4.1. Composition of doughs supplemented with oat lipid

Dough component	Dough No. 1	Dough No. 2	Dough No. 3	Dough No. 4
Reform wheat flour, g	10	10	10	10
30% NaCl, g	0.5	0.5	0.5	0.5
Oat lipid, 2133-18-1, g	0.25	0.25	0.25	0.25
Water (500 BU) (53.7%), ml	4.87	4.87	4.87	4.87
SP 972, LUT/kg flour	0	500	1000	5000

The dough was mixed at 30°C using a 50 g Brabender Farinograph. After 60 min. fermentation, the dough was frozen and freeze-dried followed by extracting lipids from the freeze-dried dough and analysing for polar lipids using HPLC. The results are summarised in Table 4.2:

Table 4.2. Content of polar lipids in doughs supplemented with oat lipids. Values are wt% of freeze-dried dough

Polar lipid component	Dough No. 1 (control)	Dough No. 2	Dough No. 3	Dough No. 4
DGDG	0.751	0.633	0.504	0.221
PC	0.301	0.209	0.159	0.055
DGMG	0.032	0.094	0.112	0.233
LPC	0.276	0.342	0.342	0.409

It appears from the above results that addition of oat lipid results in an enhanced level of both phospholipids and glycolipids and that the SP 972 lipase to a large extent utilises

DGDG and PC as substrates. Accordingly, the addition of the enzyme has a significant effect in the doughs in respect of formation of more polar lipid components.

EXAMPLE 5

Baking experiments to evaluate the possible synergistic effect of adding a cereal lipid and Lipase SP 979 to doughs

Baking experiments were carried out in which the effect of a fractionated oat lipid preparation (2133-100-1) alone and in combination with Lipase SP 979 or GRINDAMYL™ EXEL 16 on bread quality was studied to assess whether a synergistic effect of oat lipid and lipases could be demonstrated. The procedure for making the dough and baking the dough was as described above for "mini baking test". The amount of additives used and the results in respect of bread quality are summarised in Table 5.1:

Table 5.1. The effect on bread quality of oat lipid and lipases

Additives	Specific volume, ml/g	Relative volume
Control w/o additives	4.01	100
Oat lipid, 0.3%	3.91	98
Oat lipid, 0.3% + 1010 ppm SP 979	5.75	143
Oat lipid, 0.3% + 200 ppm EXEL 16	4.70	117

These baking experiments indicate a very strong volume effect by the combination of oat lipid and Lipase SP 979. The oat lipid fraction alone did not have any improving effect on bread volume. It was also very clear that the commercial lipase GRINDAMYL™ EXEL 16 although it showed a slight volume improvement effect had significantly less bread volume improvement effect than the SP 979 lipase which relative to the control without additives gave a specific volume improvement of 43% and relative to the commercial lipase an improvement of about 22%.

In addition to the improved bread volume, the addition of Lipase SP 979 resulted in improved bread crumb structure and bread appearance. This is illustrated in Fig. 2 from which it appears that bread baked with SP 979 gave a nice and tender crumb structure and a significant oven spring.

EXAMPLE 6

The effect of Lipase SP 972 alone and in combination with acylglycerols on dough quality and the quality of toast bread

Lipase SP 972 was tested alone and in combination with soy oil in baking experiments with toast bread carried out as described above, and the effects on dough characteristics and bread quality was compared with those of GRINDAMYL™ EXEL 16 and DIMODAN™ SDM-T. The results on dough quality parameters and the bread quality of these experiments are summarised in the below tables:

Table 6.1. The effect on dough extensibility and stickiness of Lipase SP 972, soy oil, shortening, GRINDAMYL™ EXEL 16 and the emulsifier, DIMODAN™ SDM-T alone and in combinations

	Dough score (0-10) after mixing		Dough score (0-10) after moulding	
Additive	Extensibility	Stickiness	Extensibility	Stickiness
Control	5	5	5	5
2% shortening	5	5	6	5
2% shortening + 500 LUT/kg SP 972	6	5	7	5
500 LUT/kg SP 972	6	5	6	5
1% soy oil	6	5	6	5
1% soy oil + 500 LUT/kg SP 972	6	5	7	5
1000 LUT/kg SP 972	6	5	7	4
1% soy oil + 1000 LUT/kg SP 972	6	5	7	4
0.4% DIMODAN™ SDM-T	6	5	6	5
200 ppm EXEL 16	6	5	7	5
2% shortening + 200 ppm EXEL 16	6	5	7	4
1% soy oil + 200 ppm EXEL 16	6	5	7	4

Table 6.2. The effect on specific volume, crumb quality and softness of Lipase SP 972, soy oil, shortening, GRINDAMYL™ EXEL 16 and the emulsifier, DIMODAN™ SDM-T alone and in combinations

Additive	Specific volume	Crumb score (0-10)	Softness, day 3	Softness, day 7
Control	3.72	4	82	129
2% shortening	3.98	7	57	91
2% shortening + 500 LUT/kg SP 972	4.63	3	48	69
500 LUT/kg SP 972	4.26	7	45	75
1% soy oil	4.05	1	83	96
1% soy oil + 500 LUT/kg SP 972	4.65	5	48	77
1000 LUT/kg SP 972	4.01	7	44	70
1% soy oil + 1000 LUT/kg SP 972	3.29	10	100	135
0.4% DIMODAN™ SDM-T	4.17	7	58	89
200 ppm EXEL 16	3.25	7	108	146
2% shortening + 200 ppm EXEL 16	3.87	6	55	86
1% soy oil + 200 ppm EXEL 16	4.12	4	73	104

These baking experiments confirmed that the addition of Lipase SP 972 alone and in combination with acylglycerol lipids resulted in a significant improvement of bread volume and softness and this effect was clearly better than that of the commercial lipase. GRINDAMYL™ EXEL 16. Additionally, as it appears from the above tables, the addition of Lipase SP 972 gave improved dough extensibility and crumb score. It is also evident from the above results that SP 972 gave very positive effects in combination with both soy oil and shortening.

In contrast to the effect of Lipase SP 972, the addition of GRINDAMYL™ EXEL 16 did not result in improvement of bread softness.

EXAMPLE 7

The effect on dough characteristics and bread quality of Lipase SP 979

Lipase SP 979 which has relatively low activity on short chain triglycerides but is particularly active towards glycerides with long chain fatty acids, and towards phospholipids was tested in toast bread baking experiments. The procedure for making the toast bread was as described above, but with the addition of additives as indicated in the following Table 7.1 which summarises the results of the experiments in respect of dough and bread quality.

Table 7.1. The effect of Lipase SP 972 alone and in combination with lecithin or soy oil on dough and bread quality

	Dough score after moulding		Specific volume	Crumb score	Softness day 1	Softness day 3	Softness day 7
	Extensibility	Stickiness					
Control	5	5	4.4	3	37	69	103
500 PLU/kg SP 979	6	5	4.27	8	32	59	90
1000 PLU/kg SP 979	8	5	5.19	8	20	42	61
2000 PLU/kg SP 979	8	5	4.84	7	21	39	57
0.5% lecithin	6	5	4.82	6	25	52	76
500 PLU/kg SP 979 + 0.5% lecithin	6	5	4.43	8	31	58	87
1000 PLU/kg SP 979 + 0.5% lecithin	8	5	5.21	8	19	38	60
2000 PLU/kg SP 979 + 0.5% lecithin	8	5	4.88	9	20	38	58
500 PLU/kg SP 979 + 1% soy oil	8	5	4.8	8	24	38	69
1000 PLU/kg SP 979 + 1% soy oil	6	5	4.91	9	24	41	67
2000 PLU/kg SP 979 + 1% soy oil	6	5	4.33	6	34	56	88
0.4% DIMODAN SDM-T	6	5	4.44	8	31	55	86

These baking experiments show a very interesting effect of Lipase SP 979 in terms of improved bread volume and crumb structure. This effect is significantly better than that of the commercial emulsifier, DIMODAN™ SDM-T.

Even more interesting, however, is the effect of this enzyme on bread softness which clearly indicates that Lipase SP 979 both alone and in combination with acylglycerols and phospholipids has a significant softness improving effect. It was observed that the crumb of the bread made in this experiment with addition of SP 979 was moist and that the

crumb structure of such bread was very homogeneous and had a bite which was shorter than that of the control bread without additives.

EXAMPLE 8

The effect of Lipase SP 979 alone and in combination with oat lipid on bread quality

Mini bread baking experiments were carried out according to the procedures described above, but with the additions of additives as indicated in the below Table 8.1 in which the effects on specific volume and formation of free fatty acids (analysed colourimetrically) in the doughs are summarised:

Table 8.1. The effect of Lipase 979 on bread volume and formation of free fatty acids

	Specific volume	wt% free fatty acids
Control	3.53	0.241
0.3% oat lipid 2133-100-1	3.72	0.243
0.3% oat lipid + 1010 ppm SP 979	5.45	0.416
0.3% oat lipid + 200 ppm EXEL 16	4.5	0.377
1010 ppm SP 979	4.84	0.338
200 ppm EXEL 16	4.08	0.317

The above results demonstrate a significant dough strengthening effect of Lipase SP 979 as reflected in enhanced specific volume and crumb structure and appearance. The effect of SP 979 was even more pronounced when it is combined with oat oil.

Proofed doughs from this baking test were freeze-dried and lipids extracted and subjected to HPLC analysis. The results of this analysis are shown in Table 8.2:

Table 8.2. The effect of Lipase SP 979 on the hydrolysis of glycolipids and phospholipids in dough (values are in wt%)

	DGDG	MGDG	PC	LPC
Control	0.176	0.033	0.025	0.193
0.3% oat lipid 2133-100-1	0.190	0.031	0.036	0.224

0.3% oat lipid + 1010 ppm SP 979	0.089	0.020	0.006	0.228
0.3% oat lipid + 200 ppm EXEL 16	0.188	0.031	0.038	0.197
1010 ppm SP 979	0.061	0.018	0.007	0.114
200 ppm EXEL 16	0.203	0.039	0.032	0.229

It appears from these results that Lipase SP 979 is capable of hydrolysing both glycolipids (DGDG) and phospholipids (PC). In fact more than 50% of DGDG and PC present is hydrolysed by the addition of SP 979.

EXAMPLE 9

Dough and bread quality improving effects of Lipase SP 972 and Lipase SP 979 in comparison with the commercial lipases, GRINDAMYL™ EXEL 16 and Stalingase™

The lipid hydrolysing effects of Lipase SP 972 and Lipase SP 979 were compared with those of two commercial lipases, GRINDAMYL™ EXEL 16 and Stalingase™ (Gist-brocades) (the latter enzyme is also referred to as # 1867) in a model dough system. Doughs were kept for 1 hour at 32°C and freeze-dried and polar lipids analysed by HPLC and nonpolar lipids by GLC analyses.

The model doughs had the following compositions:

Table 9.1. Composition of model doughs

	Dough No.1	Dough No.2	Dough No.3	Dough No.4	Dough No.5
Flour, Reform, g	50	50	50	50	50
30% NaCl, g	2.5	2.5	2.5	2.5	2.5
Water, 500 BU, 53.7%, ml	24.35	24.35	24.35	24.35	24.35
SP 972, LUT/kg flour		2000			
SP 979, PLU/kg flour			1000		
EXEL 16, ppm				200	
Stalingase™, ppm					200

The content of free fatty acids and triglycerides (GLC analysis) and the polar lipids, DGDG and PC (HPLC analysis) in the doughs are summarised in Table 9.2:

Table 9.2. Content of free fatty acids, triglycerides and polar lipids in model doughs (values are in wt%

	Free fatty acids	Triglycerides	DGDG	PC
Control	0.188	0.378	0.204	0.023
2000 LUT SP 972	0.419	0.251	0.026	0.002
1000 PLU SP 979	0.285	0.359	0.153	0.009
200 ppm EXEL 16	0.257	0.321	0.224	0.022
200 ppm Stalingase™	0.285	0.276	0.217	0.024

Based on these values it is possible to calculate the amount of free fatty acid (FFA) formed in the dough and the amounts of triglycerides, DGDG and PC, respectively that are hydrolysed in the doughs. These data are shown in Table 9.3:

Table 9.3. Formation of free fatty acids and hydrolysis of triglycerides, DGDG and PC in model doughs (wt%)

	FFA formed	DGDG hydro-lysed	PC hydrolysed	Triglycerides hydrolysed
Control	0	0	0	0
2000 LUT SP 972	0.230	0.178	0.021	0.127
1000 PLU SP 979	0.097	0.051	0.014	0.019
200 ppm EXEL 16	0.069	-0.020	0.001	0.057
200 ppm Stalingase™	0.097	-0.013	-0.001	0.102

When the activity of a lipase in a dough is expressed in terms of the amount of FFA formed in the dough as shown in the above table, it is clearly demonstrated that Lipase SP 972 and Lipase SP 979 are very active in respect of hydrolysis of both glycolipids (DGDG) and phospholipids (PC) as compared to both of the commercial lipases tested, which show no effect at all on these substrates.

With respect to effect on triglycerides, Lipase SP 972 and Lipase SP 979 are both active, however to a less extent than the tested commercial lipases.

Accordingly, these results confirm that the two lipases according to the invention hydrolyse a wide range of lipid substrates including nonpolar lipids such as triglycerides and polar lipids such as the glycolipid, DGDG, and the phospholipid, PC.

The data summarised in Table 9.3 may also be illustrated graphically to show the relationship between the ability of enzymes to hydrolyse triglycerides and the ability to hydrolyse glycolipids (Fig. 3) and the relationship between the ability of enzymes to hydrolyse triglycerides and the ability to hydrolyse phospholipids (Fig. 4). It appears from these figures that this relationship for Lipase SP 972 and Lipase SP 979, respectively in respect of DGDG can be described as a curve having slopes of 1.3965 and 2.6405, respectively and the corresponding values in respect of PC are 0.1648 and 0.7248, respectively.

These data clearly illustrate the relatively high activity of Lipase SP 972 and Lipase SP 979 on glycolipids and phospholipids in relation to their effect on triglycerides.

CLAIMS

1. A method of preparing a flour dough, said method comprising adding to the dough components an enzyme that, under dough conditions, is capable of hydrolysing a nonpolar lipid, a glycolipid and a phospholipid, or a composition containing said enzyme, and mixing the dough components to obtain the dough.

2. A method according to claim 1 wherein at least one of the nonpolar lipid, the glycolipid and the phospholipid is a naturally occurring lipid component occurring in the flour used for the dough.

3. A method according to claim 2 wherein the naturally occurring lipid is a phospholipid.

4. A method according to claim 3 wherein the phospholipid is phosphatidylcholine (PC)

5. A method according to claim 2 wherein the naturally occurring lipid is a glycolipid.

6. A method according to claim 5 wherein the glycolipid is digalactosyldiglycerids (DGDG).

7. A method according to claim 1 wherein at least one of the nonpolar lipid, the glycolipid and the phospholipid is added to the dough.

8. A method according to claim 7 wherein the nonpolar lipid being added is an acylglycerol.

9. A method according to claim 8 wherein the acylglycerol being added is selected from the group consisting of a vegetable oil, a vegetable fat, an animal oil, an animal fat, shortening and butter.

10. A method according to claim 9 wherein the vegetable oil is a naturally occurring cereal oil including oat oil.

11. A method according to claim 7 wherein the polar lipid being added is a phospholipid selected from the group consisting of phosphatidylinositol (PI), phosphatidylglycerol (PG), phosphatidylcholine (PC) and phosphatidylethanolamine (PE).

12. A method according to claim 1 wherein the dough is a yeast leavened dough.

13. A method according to claim 1 wherein the enzyme is added in amount which is in the range of 10 to 100,000 LUT/kg flour or in the range of 10 to 100,000 PLU/kg flour.

14. A method according to claim 13 wherein the amount of enzyme is in the range of 100 to 10,000 LUT/kg flour or 100 to 10,000 PLU/kg flour.

15. A method according to claim 1 wherein the dough is a bread dough, the method comprising as a further step that the dough is baked to obtain a baked product.

16. A method according to claim 1 wherein the dough is a dough selected from the group consisting of a pasta dough, a noodle dough and a cake dough or batter.

17. A method according to claim 1 wherein the enzyme is added in an amount that results in an increase of the specific volume of the baked product that is at least 10%, relative to a baked product made under identical conditions except that the enzyme is not added.

18. A method according to claim 1 wherein a further enzyme is added to the dough.

19. A method according to claim 18 wherein the further enzyme is selected from the group consisting of a lipase, a starch degrading enzyme, a hemicellulase, a cellulase and an oxidoreductase.

20. A method according to claim 1 wherein at least 25% of DGDG initially present in the dough is hydrolysed.

21. A method according to claim 1 or 20 wherein at least 25% of PC initially present in the dough is hydrolysed.

22. A method according to claim 1 wherein the enzyme is characterised in that the

relationship between the ability of the enzyme tohydrolyse triglycerides and the ability to hydrolyse glycolipids can be described as a curve having a slope which is at least 1.0.

23. A method according to claim 1 wherein the enzyme is characterised in that the relationship between the ability of the enzyme to hydrolyse triglycerides and the ability to hydrolyse phospholipids can be described as a curve having a slope which is at least 0.1.

24. A dough improving composition comprising an enzyme that, under dough conditions, is capable of hydrolysing a nonpolar lipid, a glycolipid and a phospholipid, and optionally at least one further dough component.

25. A composition according to claim 24 that comprises a further enzyme selected from the group consisting of a lipase, a starch degrading enzyme, a hemicellulase, a cellulase and an oxidoreductase.

26. A composition according to claim 24 where the further dough component is selected from the group consisting of a cereal flour, yeast, a chemical leavening agent, a dough strengthening agent, an emulsifier, a sugar, an acylglycerol, a phospholipid, aglycolipid and a salt.

27. A method according to claim 1 wherein the enzyme is added in a composition according to any of claims 24-26.

28. A dough obtained by the method of any of claims 1-21 and 27.

29. A dough according to claim 28 which is frozen or packaged in a controlled atmosphere.

30. A baked product that is obtained by baking the dough of claim 28.

31. A noodle product made from the dough of claim 28.

32. A pasta product made from the dough of claim 28.

METHOD OF IMPROVING DOUGH AND BREAD QUALITY

ABSTRACT

Method of preparing a dough, the method comprising adding to the dough an enzyme that is capable of simultaneously hydrolysing a nonpolar lipid, a glycolipid and a phospholipid. One or more substrates for the enzyme may be added, e.g. galactolipids such as digalactodiglyceride (DGDG) or phospholipids, e.g. phosphatidyl choline (PC). The lipid substrates can be added to the dough in the form of cereal lipids such as oat oil. The method provides doughs with improved extensibility and reduced stickiness, and baked bread products with high specific volume, improved softness and excellent crumb structure.

Fig. 1

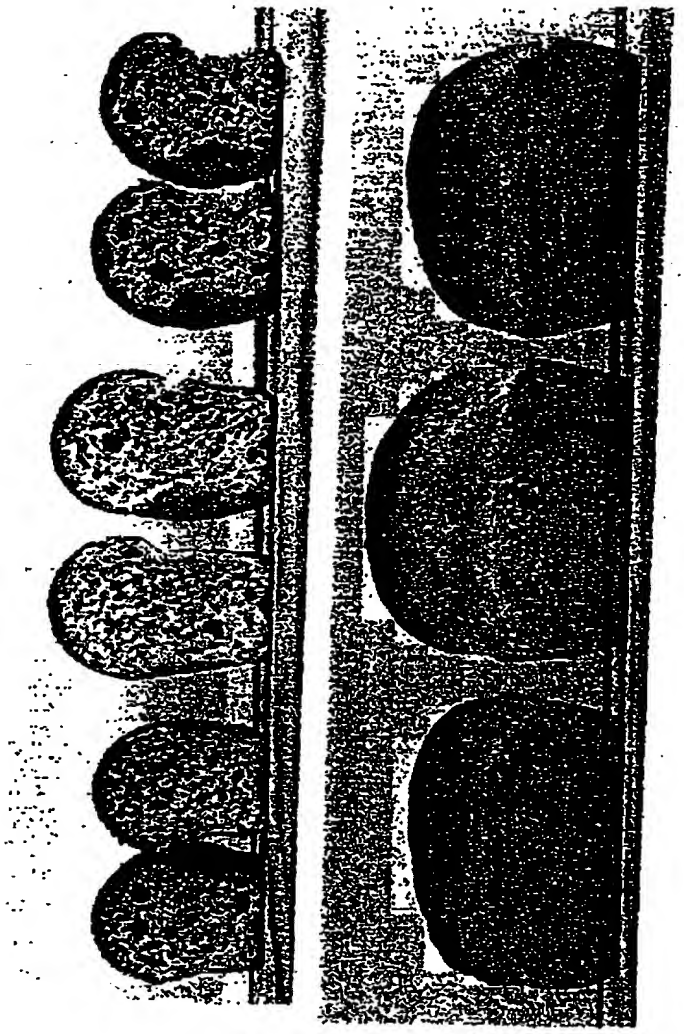

Fig. 2

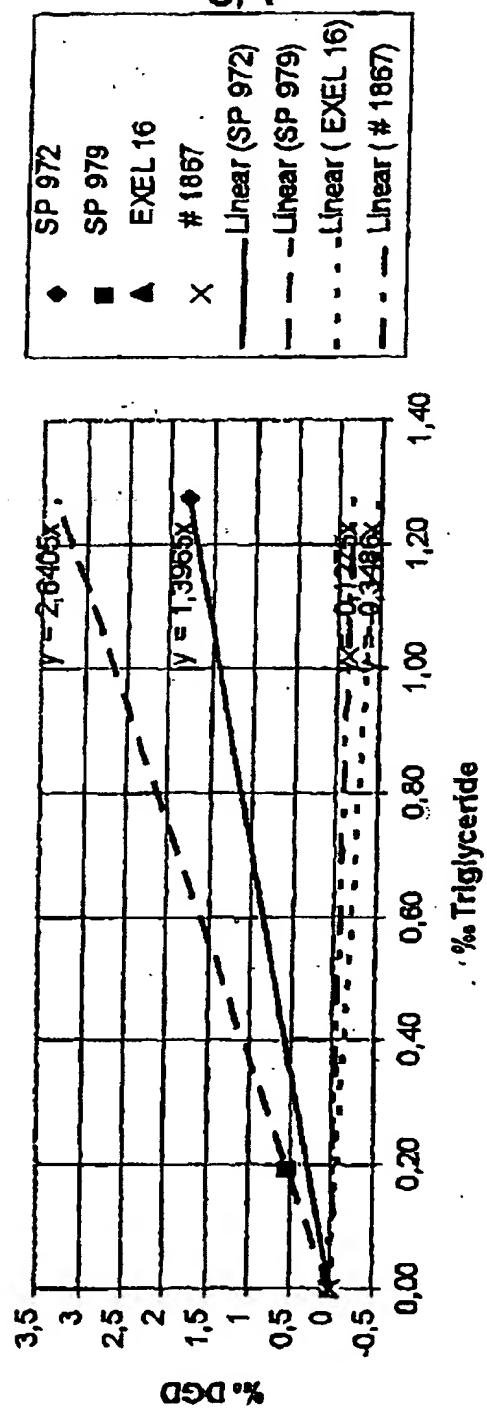

Fig. 3

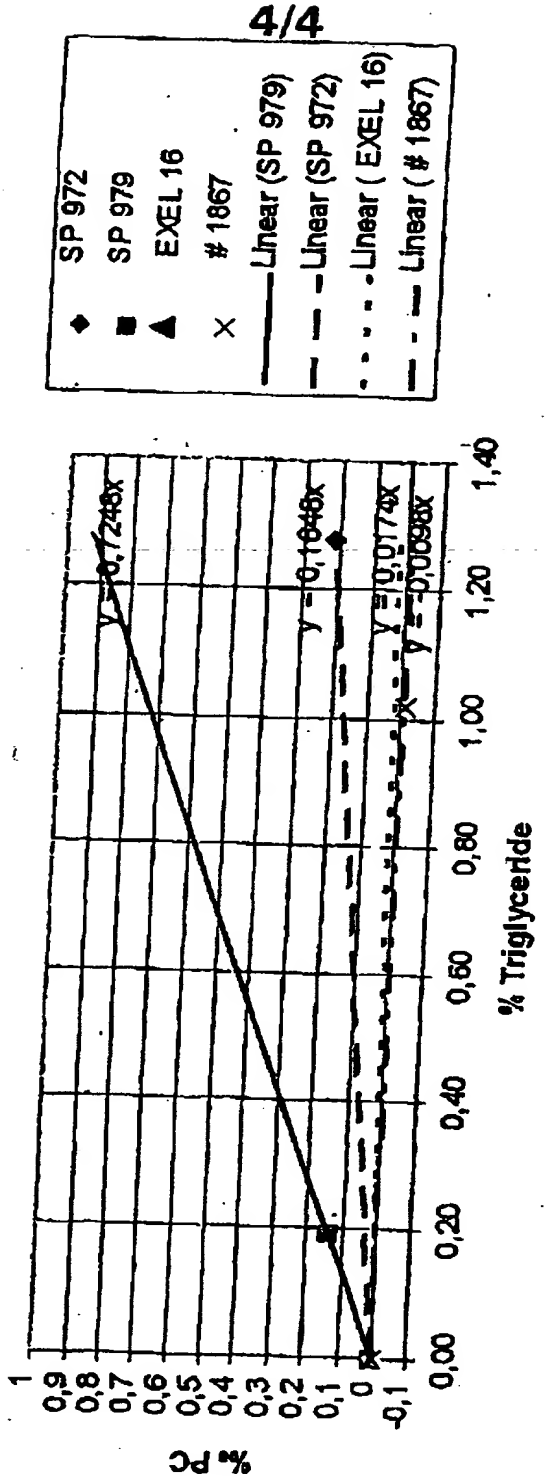

Fig. 4